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### Making the invisible visible

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*Published in:*  
Seminars in Cell & Developmental Biology

*DOI:*  
[10.1016/j.semcd.2016.02.013](https://doi.org/10.1016/j.semcd.2016.02.013)

*Publication date:*  
2016

*Licence:*  
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*Document Version*  
Peer reviewed version

[Link to publication in Discovery Research Portal](#)

*Citation for published version (APA):*  
van Steensel, M. A. M. (2016). Making the invisible visible. *Seminars in Cell & Developmental Biology*, 52, 58-65. <https://doi.org/10.1016/j.semcd.2016.02.013>

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# Making the invisible visible

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## Abstract

In this review, I will discuss how careful scrutiny of genetic skin disorders could help us to understand human biology. Like other organs, the skin and its appendages, such as hairs and teeth, experience fundamental biological processes ranging from lipid metabolism to vesicular transport and cellular migration. However, in contrast to other organ systems, they are accessible and can be studied with relative ease. By visually revealing the functional consequences of single gene defects, genetic skin diseases offer a unique opportunity to study human biology. Here, I will illustrate this concept by discussing how human genetic disorders of skin pigmentation reflect the mechanisms underlying this complex and vital process.

Key words: skin, genodermatosis, genetic, pigment, pigmentation

## Introduction

Genetic disorders offer a unique window onto human biology, offering a glimpse of the machinery that underlies the normal functioning of our bodies. By correlating clinical observations in patients with inherited conditions and knowledge obtained from work on

model organisms such as mouse or zebrafish, we can begin to understand the molecular underpinnings of health and disease.

Making the correlation with human biology, however, is hampered by the poor accessibility of the study subject. To properly analyse liver disease, for example, surgical procedures such as biopsies would be required. Understandably, these are only warranted if there is a clear medical need. Samples from other organs, such as the brain, are even more difficult, if not impossible, to obtain. As a consequence, we have accumulated vast knowledge about our model organisms that we do not know how to apply to our own physiology, if it can be done at all. Fortunately, there is a way out of this conundrum.

The skin is the largest organ in the human body and, at the same time, by far the most accessible. Simple visual inspection can reveal much about cutaneous health already, and samples for analysis of arbitrary sophistication can be easily obtained with a minimum of discomfort to the donor. Moreover, the skin hosts an impressive array of associated structures such as blood vessels, nerves and mini-organs such as hair follicles that have a complex life of their own and are easily sampled. Add in a highly diverse microbiota consisting of eukaryotes, prokaryotes and viruses, and it becomes clear that all of biology is being played out on the canvas of the skin, whilst being quite accessible to scientific enquiry.

By showing us what happens in the context of a single, well defined gene defect, genetic skin disorders (genodermatoses) offer a unique opportunity to deeply study basic biological processes. In this review, I will discuss this concept. Rather than elaborating on individual disorders, however, I will show how a particular symptom in genetic disorders, in this case abnormal cutaneous pigmentation, reflects the underlying pathology, and what that tells us about healthy skin biology. Of note, pigment-based skin discolourations are

extremely common in the general population, which is suggestive that such blemishes share common mechanisms with the genetic diseases of which they can be part.

## **Pigmentation**

The ability of mammalian skin to tan upon exposure to sunlight is a crucial component of its defense against the damaging effects of UV-radiation. Specialised, neural-crest derived melanocytes produce two major types of pigment: eumelanin and pheomelanin.

Eumelanins (black and brown variants exist) imparts a darker color than red and yellow pheomelanin, which is responsible for red to pink hues, as in lips or nipples in people with a Fitzpatrick type I-II (light Caucasian) skin [1] . There is a third type called neuromelanin, which is produced in the brain by catecholaminergic neurons of the substantia nigra and locus coeruleus. Its function there remains elusive, but it is known to have complex roles in local immunomodulation and protection from oxidative stress. Loss of neuromelanin is associated with Parkinson's disease [2] .

Melanin synthesis is a highly complex, multistep process that requires more than 190 genes and is tightly regulated. Upon UVB irradiation, keratinocytes initiate pigment formation in melanocytes by producing alpha-melanocyte stimulating hormone ( $\alpha$ -MSH) in a p53-dependent manner [3]. As an aside,  $\alpha$ -MSH is produced from a precursor called pro-opiomelanocortin (POMC), whose other products include ACTH and  $\beta$ -endorphin. The latter is an endogenous opioid and it is responsible for the addictive effects of suntanning [4]. One wonders about the evolutionary pressures that shaped this particular system, which effectively rewards pale-skinned individuals for seeking sufficient UVB exposure to damage keratinocyte DNA.  $\alpha$ -MSH activates the melanocortin 1 receptor (MC1R), which in turn activates the transcription factor MITF that initiates the expression of a wide range of genes involved in melanocyte migration and survival, as well as melanin production and

transport [5]. Hypomorphic *MC1R* alleles are associated with red hair, fair skin and freckling and predispose to the development of melanoma, a malignant and highly invasive melanocyte tumor (OMIM #266300; this number refers to an entry in the Online Mendelian Inheritance in Man database at <http://www.ncbi.nlm.nih.gov/omim>).

Melanins usually are co-polymers of eumelanin and pheomelanin, deposited onto protein fibrils in a specialised membranous organelle, the melanosome. When mature, these are exocytosed by melanocytes, to be actively internalised by keratinocytes in an incompletely understood process that depends on a number of small G proteins including RAB11B, RAB27A, RAB32 and RAB38 [6] . Keratinocytes in the basal layers of the epidermis need to have their nuclei protected from UVB, as they comprise the cells that are responsible for renewing most of the epidermis while its cells are being shed due to normal differentiation. This protection is provided by the so-called keratinocyte microparasol, a perinuclear microtubule-melanosome complex that is most prominent in the basal layer. This structure needs to be actively maintained, and requires the activity of dynein motor proteins [7] .

Thus, pigmentation is a highly complex and active process, much of which remains poorly understood. Fortunately, there are genetic disorders that can bring some light to this darkness and they will be discussed here in some detail. This review will concern itself with skin pigmentation only, even though the diseases that are discussed can also cause ocular albinism, i.e., loss of pigment in the iris and retina.

## **Human diseases reveal the machinery of pigment production**

A complete or partial lack of pigmentation is referred to as albinism and can affect all structures that contain pigment including the iris and retina of the eye, as in oculocutaneous albinism. Several forms of albinism exist and many of the underlying gene defects have now been elucidated. Collectively, these reveal the critical steps in

pigmentation and show that it depends on a large number of quite diverse processes, ranging from cell fate determination to vesicle maturation and protein transport. Figure 2 summarises these complex events and illustrates the relationship between the disorders resulting from their dysfunction.

### **Neural crest specification and migration**

Melanocytes are derived from the neural crest, a population of cells that arise from the margins of the neural epithelium early in embryogenesis and migrate extensively to give rise to structures such as the facial skeleton [8] . Genetic events that affect neural crest migration and differentiation, therefore, can be expected to affect not only pigmentation, but also facial development and hearing. Indeed, several human disorders were identified that show precisely such phenotypes. They have been instrumental in understanding how neural crest migration and fate determination are controlled, and what structures it contributes to.

The classic example is Waardenburg syndrome type 1 (#193500), caused by heterozygous mutations in the paired homeobox gene *PAX3* [9]. People with this autosomal dominant disorder have a very distinct phenotype consisting of a white forelock, pale blue, eyes that seem wide-set because the inner corners of the eyes are too far apart (dystopia canthorum) and sensory hearing loss. These abnormalities result from partially defective neural crest migration, which *PAX3* regulates through *MITF* and the transcription factor *SOX10* that transactivates *MITF* [10,11]. Accordingly, heterozygous mutations in *MITF* cause Waardenburg syndrome type 2A (WS2A, #193510 - the number indicates a particular phenotype, the letter a distinct causative gene), which is like WS1 with the exception of the dystopia canthorum [12]. As expected from *PAX3*'s interactions, mutations in *SOX10* can cause a WS2 phenotype as well; WS2E (#611584) was identified in 2009 in a boy who had the WS2 phenotype plus neurological manifestations, including

hypomyelination and absence of cochlear nerves and olfactory bulbs [13]. More damaging heterozygous mutations in *SOX10* cause WS type 4C (#613266) which in addition to the manifestations of WS2C is associated with Hirschsprung's disease [14] .

Additional phenotypic and genetic heterogeneity in Waardenburg syndrome reveals additional factors that are required for proper neural crest migration and specification and which, notably, all interact with MITF in one way or another. WS2 type D (#608890) is caused by homozygous deletions of the *SNAI2* gene, which codes for a Slug-related transcription factor whose expression can be regulated by MITF [15]. WS type 4A (#277580) is associated with mutations in the Endothelin-B receptor (*EDNRB*), and type 4B with mutations in one of its ligands, Endothelin 3 (*EDN3*, #613265) [16,17]. *EDNRB* can regulate MITF activity via the MAPK pathway [18]. Thus, human pathology is elegantly delineating an intricate signaling network that has MITF at its core. Since MITF activity is additionally stimulated through the receptor tyrosine kinase KIT, one would predict to find Waardenburg syndrome associated with defective KIT action. And indeed, very recently a heterozygous, probably dominantly acting, missense mutation in *KITLG* coding for KIT ligand was observed to segregate with a WS2 phenotype [19].

There are other human neural crest disorders that show a clear pigmentary phenotype, although in many of those diseases that is usually not noticed because other syndrome manifestations, such as cardiovascular malformations, take center stage. Examples include the Borrone dermato-cardio-skeletal syndrome (BDCS, #211170) that is caused by homozygous mutations in *SH3PXD2B*, which codes for an adapter protein required for full podosome functionality [20]. Podosomes are actin-based structures capable of digesting extracellular matrix, made by many different cells when they need to move. Tumor invasion and metastasis likewise are mediated by podosome-like structures that are sometimes referred to as invadopodia [21]. In Borrone syndrome and the allelic disorder

Frank-Ter Haar syndrome (#249420), patients show clear neural crest-related phenotypes [22]. Notably, they have scattered areas of abnormal pigmentation on their skin, which strongly suggests that melanocyte progenitor cells they need to digest and remodel the extracellular matrix with podosomes to migrate to their destinations. This is an aspect of neural crest cell biology that remains to be explored, but is of obvious importance for understanding why malignant melanocytes are so invasive.

### **Melanin synthesis**

Melanin is synthesized from the amino acid tyrosine in a series of reactions that critically depend on the activity of three known enzymes, two of which are associated with human disease. In these disorders, the capacity for melanin synthesis is strikingly evident in the amount and nature of skin pigmentation.

Tyrosinase crucially catalyses the conversion of tyrosine to DOPAquinone, the precursor for both eumelanin and pheomelanin. Complete loss of tyrosinase activity causes oculocutaneous albinism type 1 (OCA1A, #203100), an autosomal recessive disorder in which pigment is completely absent from the integument and the eyes [23]. A further defining characteristic is misrouting of the optic nerves, which manifests outwardly in the eyes moving to and fro rhythmically, a movement known as nystagmus. This phenomenon is seen in many types of oculocutaneous albinism.

In people with reduced tyrosinase activity, logically, some pigment will still be formed and they consequently have the so-called “yellow” type of albinism (OCA1B, #606952) [24].

The production of eumelanin requires the activity of DCT and TYRP1 [1]. The former has no associated disease, but loss of TYRP1 activity causes “rufous” oculocutaneous albinism (OCA3, #203290), which was first reported in South Africa amongst individuals



with a type 5 (very dark) skin [25]. In those people, OCA3 manifests with a bright copper-red coloration to the skin and hair, as they can still make pheomelanin and tyrosinase can also catalyse some eumelanin production. TYRP1's function in human melanogenesis is not known. Its mouse ortholog, mutations in which cause the *brown* phenotype, was identified as having dopachrome tautomerase activity [26]. The human version may not have that function [27].

## **Melanosomes**

Melanin synthesis and deposition take place in melanosomes, which are large ( $\pm 500$  nm in diameter) endosome-derived organelles. Because of their size, they are easily visualised with a brightfield microscope and have consequently been well studied, in particular in mouse coat color mutants. These have proven invaluable in teasing out the mechanisms of melanosome maturation. Melanosome biogenesis and maturation are intimately linked with melanin synthesis and proceed through four stages. Although these are well defined, their molecular underpinnings are still incompletely understood. At each stage, appropriate melanosome components and enzymes for melanin synthesis must be delivered, requiring intricate protein sorting and transport steps. Several excellent reviews discuss this process in great detail (see for instance [28,29]); the present discussion focuses on known and well-understood connections with human disease.

As it turns out, defective melanosome maturation underlies several human disorders in which altered pigmentation can be accompanied by various, often quite severe hematological and/or immunological abnormalities in addition to neurological defects. This association might seem surprising, until one realises that platelet dense granules and secretory granules of cells such as granulocytes and cytotoxic T-lymphocytes are also lysosome-related [30]. Intriguingly, in these melanosome maturation defects, pigmentation is often not completely absent. Rather, it is diluted, resulting for instance in silvery rather

than white or light-blond hair (figure 3). When identified in a patient, this highly distinctive phenomenon should prompt thorough clinical evaluation for associated immune deficiencies or bleeding tendency as well as neurological issues.

### **Melanosome maturation and transport**

Stage I, unpigmented melanosomes are vacuolar endosomes. They contain the protein PMEL, which causes intraluminal vesicles to form the amyloid fibrils that characterise stage II melanosomes [31]. These fibrils, an apparently physiological form of amyloid, provide a scaffold for melanin to polymerise and are thought to protect melanocytes from the toxic intermediates of its synthesis. PMEL17 mutations have not been reported in humans, but are responsible for the mouse *silver* phenotype and the merle coat pattern that is prized in several dog breeds [32,33]. In both animal species, the gene defect causes hearing loss and eye abnormalities. How PMEL17 is sorted to the endosomes remains to be determined. Stage II melanosomes are characterised by internal striations, which are the fibrils made by PMEL17 and which form the scaffold for melanin synthesis, which takes place in stage III. In order for the melanosome to proceed to that stage, it needs to take delivery of the melanin-synthesizing machinery, including tyrosinase, DCT and TYRPI, through tubular-endosomal transport from the trans-Golgi compartment (reviewed in [28]). This process depends on the adapter proteins AP1 and AP3. Loss of the  $\beta$ 3A subunit of AP3 causes Hermansky-Pudlak syndrome type 2 (HPS2, #608233), in which oculocutaneous albinism is associated with immunodeficiency and prolonged bleeding [34]. The immune defect results from AP3 having roles in various trafficking events in antigen-presenting cells and cytotoxic T-lymphocytes (reviewed in [35]). Patients are not completely devoid of pigmentation, which indicates that there is some redundancy in the system - there must be other paths to deliver material to the maturing melanosomes.

Indeed, HPS types 1 (#) and 4 (#) were found to be caused by mutations in components of the lysosomal complex BLOC-3, which acts as a guanine-nucleotide exchange factor to RAB32 and RAB38 [36,37]. These two small GTPases are thought to facilitate targeting of tubular connections to the maturing melanosome, which also requires the lysosomal complexes, BLOC-1 and -2 [38,39]. Like BLOC-3, they are implicated in HPS. Analysis of melanocytes from a patient with HPS9, which is due to mutations in the Pallidin subunit of BLOC-1, showed that TYRP1 was not being sorted to melanosomes correctly [40]. TYRP1 is likewise absent from maturing melanosomes in BLOC-2 deficient melanocytes derived from HPS (type 5) patients [41].

Thus, major determinants of melanosome biogenesis are revealed by the HPS phenotype. All types of HPS are associated with prolonged bleeding, revealing profound and intriguing parallels between melanogenesis and dense granule biogenesis. However, depending upon additional functions that the causative gene might have, they may also have distinct clinical manifestations as in HPS2 (includes immunodeficiency) or in HPS1/4, which include pulmonary fibrosis because the BLOC-3 complex also has a role in surfactant secretion in alveolar type II cells [42]. There is no doubt that additional, genetically distinct HPS subtypes will be discovered in the future, since at least 15 murine equivalents of Hermansky-Pudlak syndrome have been identified.

### **Melanosome delivery to the cell membrane**

Whereas stage 3 critically depends on protein sorting and vesicular transport events, stage 4 has no such requirements. Instead, this fully mature melanosome, distinguished by its lack of internal structure, needs to be transported into melanocyte dendrites, which are cellular extensions that contact up to 40 keratinocytes. Transport into those structures takes place along the microtubules, presumably mediated by kinesin motors, although there is no clinical evidence for this and the available *in vitro* data are conflicting. We do

know that melanosomes switch tracks once they reach the dendrite trips, moving from the microtubule onto the actin cytoskeleton where they are captured by a complex consisting of the unconventional myosin MYO5A, the small G protein RAB27A and the exophilin Melanophilin (MLPH) [43]. Loss-of-function MYO5A mutations cause Griscelli syndrome type 1 (#214450) [44]. People with this rare, autosomal recessive disorder have silvery hair, light skin and neurological dysfunction including muscular hypotonia and seizures. From studying GS type 2 (#607624, figure 3) and 3 (#609227), which are characterised by additional immune deficiency, or just hypopigmentation respectively, RAB27A and MLPH were identified [45,46]. The lack of neurological symptoms and immune dysfunction in GS3 suggests that MLPH's function is specific to melanocytes and some recent data support this notion by showing that it increases the melanosome's dwell time on cortical actin. By slowing the melanosome down, MLPH probably facilitates the interactions that are required for exocytosis [47]. Interestingly, there is no ocular albinism in the various types of GS, suggesting that eye pigmentation uses different systems to transport mature melanosomes.

### **Pigment transfer**

Recent evidence suggests that melanocytes transfer their products by shedding vesicles containing melanosomes from filopodia on their dendrites [48]. These vesicles are captured by microvilli on keratinocytes and subsequently phagocytosed in a PAR22 dependent manner, after which they are degraded. The melanosomes then spread along the keratinocyte microparasol. At present, there are no known human genetic diseases associated with this phase of pigmentation. However, a number of proteins associated with pigmentation disorders do not have a known function as yet and some might be involved in pigment transfer.

### **Loose ends**

From clinical observations, we know of some proteins that are involved in pigmentation, but that do not have a clearly assigned function yet.

Type 2 oculocutaneous albinism (OCA2, #203200) is an autosomal recessively inherited disorder, in which patients retain some tanning ability that improves with age. It is caused by mutations in the *P* gene, which codes for a 12-pass transmembrane protein that is present in melanosome membranes from stage 2 [49]. Its function is unknown, but it is thought to be an anion transporter that controls melanosomal pH [50]. The molecular basis of OCA type 4 (#606574) provides strong evidence that this idea might be correct.

Mutations in *SLC45A2* cause OCA4 [51]. This gene codes for a solute carrier (MATP), which is expressed in melanosomes. Interestingly, its knockdown causes melanosome pH to drop and is associated with lowered tyrosinase activity, which can be recovered by copper treatment. Tyrosinase activity requires copper, which it binds in a pH-dependent manner [52]. Thus, MATP could be regulating tyrosinase activity by helping to control melanosomal pH, although it is unlikely to be its primary function. Rather, MATP might export as yet unknown sugars from the melanosome, as it strongly resembles known sucrose/proton symporters that use proton gradients to pump their cargo across cell or organelle membranes. What sugars would be present in melanosomes, and what their function would be remains to be investigated. It is conceivable that they could originate from the turnover of glycosylated proteins.

The importance of melanosome pH regulation presents an interesting parallel with lysosomes, whose proper function likewise depends on maintaining their internal pH within a defined (acidic) range. Indeed, in the past, melanosomes were considered as modified lysosomes, but this idea, as we've seen, is too simplistic. Rather, the two organelles share a common endosomal origin as suggested by the finding that Chédiak-Higashi syndrome (CHS, #214500) is caused by loss of the protein LYST (Lysosomal trafficking regulator)

[53] . CHS is characterised by oculocutaneous albinism, prolonged bleeding, severe immunodeficiency, recurrent bacterial infection, neurologic dysfunction and hemophagocytic lymphohistiocytosis. LYST function remains to be fully defined, but in retinal pigment epithelium from CHS patients, enlarged melanosomes were observed [54]. Interestingly, other cell types in CHS also exhibit enlarged, lysosome-like organelles. They are defective in secretory lysosome exocytosis, and recent work indicates that LYST might be involved in trafficking of the required effector proteins [55] . It is very tempting to speculate that it might therefore be one of the missing links in the transfer of pigment from melanocytes to keratinocytes. Consistent with this notion, LYST is one of nine BEACH (BEige And Chediak-Higashi) domain proteins, all of which are hypothesized to mediate membrane fusion and fission events [56]. Considering that Chediak-Higashi syndrome is very difficult to distinguish clinically from Griscelli syndrome, the author would like to propose that LYST could interact with the MYO5-RAB27A-MLPH complex and control melanosome fusion with the melanocyte membrane.

While much of our knowledge about the biology of pigmentation is derived from the elucidation of genetic diseases, is of interest to note that several of the genes discussed above are responsible for the normal variation in skin, hair and eye pigmentation (SHEP, 11 OMIM entries).

### **Other determinants of pigmentation**

This review only scratches the surface of human pigment biology. For instance, additional complexity to melanosome biogenesis, which apparently shares some of its machinery with neurons, is suggested by the Cross oulocerebral hypopigmentation syndrome (#257800). This is an ultra-rare disorder of pigment dilution with severe neurological dysfunction and microcephaly. Given its resemblance to Griscelli type 1 syndrome, Hermansky-Pudlak and Chédiak-Higashi syndromes, it might be caused by mutations in a

component of melanosome biogenesis that functions in the MYO5 complex. No doubt there will be other conditions identified in the future that are related to melanosome biogenesis - work in model organisms such as mouse and zebrafish has identified many pigment dilution mutants for which there is no human equivalent yet. Furthermore, melanocyte growth and activity is strongly influenced by RAS signalling, which accepts a great many inputs and has pervasive effects on cellular physiology. Whilst outside the scope of this review, this system is of considerable clinical interest because its deregulated activity is a major driver of melanoma growth [57]. How RAS signalling affects pigment production, which is increased in RASopathies, remains to be defined but is of obvious interest for clinical and cosmetic dermatology.

There exist also some fascinating mosaic disorders of pigmentation, in which a genetic defect limited to the descendants of a single cell manifests as a pigmented patch of skin, or a local accumulation of melanocytes. These phenotypes reveal the existence of additional determinants of pigmentation, including receptors that seem to have a role in the migration of melanocyte precursors. Whilst outside the scope of this review, strictly speaking, it is of interest to mention blue nevi, which are dermal accumulations of melanocytes whose brown melanin appears blue at the skin surface due to the so-called Tyndall effect. These innocuous but sometimes cosmetically disturbing lesions carry the activating mutation c.548G>A,(p.Arg183Gln) in *GNAQ*, which codes for the Gq $\alpha$  subunit of Guanine nucleotide-binding (G-) proteins that transduce signals received by G-protein coupled receptors [58].

Finally, a large number of other genetic skin disorders are associated with pigmentary abnormalities other than albinism, indicating that there is even more complexity to making and maintaining melanocytes and pigment than was just discussed. A particularly interesting group is that of the reticulate hyperpigmentations, which is implicating quite

unexpected players. For instance, dominant negative mutations in the intermediate filament proteins keratin (K)5 and its obligate partner K14 cause the blistering disorder epidermolysis bullosa simplex (EBS, #131800, 131900). A specific mutation in K5 (p.Pro25Leu) causes a peculiar manifestation of this disease, called EBS with mottled pigmentation (#131960) [59,60]. Here, the skin blisters are accompanied by patchy hyperpigmentation, in particular on the limbs. Intriguingly, other keratin (K) 5 or 14 mutations are associated with quite distinct, non-blistering disorders: Dowling-Degos disease (DDD) type 1 (#179850) and Naegeli-Franceschetti-Jadassohn syndrome/dermatopathia pigmentosa reticularis (#161000/125595, figure 4) [61,62]. People with those disorders exhibit reticular hyperpigmentation, with a preference for flexures. The mechanism of hyperpigmentation is not understood - histopathological analysis of skin samples in DDD shows increased pigment deposition in basal keratinocytes, but how keratin mutations should cause this phenomenon remains unclear. K5 mutations that cause DDD1 are associated with abnormal melanosome distribution. There seems to be a specific interaction between the K5 head domain and the chaperone Hsc70, which is involved in vesicle uncoating. Loss of this interaction could conceivably impact pigment distribution [63]. K14 does not have this specific association, and as a more general mechanism it has been speculated that inflammatory signaling results from keratinocyte apoptosis induced by the keratin mutation [64,65]. This in turn might increase pigment production by melanocytes, as in post-inflammatory hyperpigmentation. Some support for this hypothesis is provided by the recent identification of a novel disorder caused by homozygous loss of function mutations in *SASH1*, which was first described as a tumour suppressor. The new phenotype consists of mottled hypo- and hyperpigmented macules on the trunk and face and reticulate hyperpigmentation of the extremities, combined with alopecia, palmoplantar keratoderma, nail dystrophy and squamous cell carcinoma [66]. Whilst originally reported as a tumour suppressor, *SASH1* has been



recently observed to function as a scaffold for TLR4 signaling, and may also have a role in regulating NF- $\kappa$ B activity thereby implicating dysregulation of inflammation in the loss-of-function phenotype [67].

However, there are other causes of DDD that point to additional determinants of pigment production and homeostasis. Heterozygous mutations in the *POFUT1* gene cause DDD type 2 (#615327). POFUT1 is a protein O-fucosyltransferase, which adds O-fucose glycans to Notch receptors and seems to be essential for proper signaling by them [68].

Recent work has started to implicate Notch signalling in melanocyte survival and migration, showing that it is required to maintain hair pigmentation by providing a survival signal for melanocytic stem cells [69]. The existence of DDD type 4 (#615696, type 3 has no associated gene defect yet) provides additional evidence that Notch is important in pigmentary homeostasis, as DDD4 is caused by heterozygous mutations in *POGLUT1* which codes for protein O-glycosyltransferase 1, an endoplasmic reticulum (ER) O-glucosyltransferase that adds glucose moieties to the transactivating NOTCH intracellular domain [70]. Why and how alterations of cutaneous Notch signalling should give rise to reticular hyperpigmentation is not clear. There are other disorders that cause similar discolourations and whose molecular basis might help to answer this question. One of the best studied is reticulate acropigmentation of Kitamura (RAK, #615537). This disorder, which for reasons unknown affects mostly people with a type IV (Asian) skin, is caused by heterozygous mutations in the *ADAM10* gene, which codes for a metalloprotease [71]. ADAM10 has many roles, but one of them is to activate Notch1 signalling [72].

The pigmentary changes in RAK affect the backs of the hands and feet. Reticulate acropigmentation of Dohi, or dyschromatosis symmetrica hereditaria (DSH, #127400) is rather similar, but can also cause hypopigmentations to appear and affects the face, in contrast to the other disorders of reticulate hyperpigmentation. Interestingly, DSH is the

consequence of mutations in *DSRAD/ADAR1*, coding for an RNA-specific adenosine deaminase that can change adenosine in double-stranded RNA to inosine [73]. While it is not immediately obvious how RNA editing would relate to pigmentation, it was recently published that loss of A can cause apoptosis in intestinal crypt cells through endoplasmic reticulum stress, followed by interferon-mediated inflammation [74]. It also is tempting to speculate, given the phenotypic similarities between RAK and DSH, that DSRAD might in some way be involved in editing transcripts of genes downstream of Notch signaling.

Why DDD, RAK and DHS give rise to reticular hyperpigmentation remains to be explained - it is not clear why some keratinocytes should be more prone to accumulating pigment than others. Perhaps there is a relation here with the known Notch involvement in embryonic patterning, and in that case studying the diseases of reticulate hyperpigmentation might teach us a lesson or two about embryonic development.

## **Conclusion**

In this review, I have endeavoured to show how highly visible human phenotypes that result from a single gene defect can illuminate very complex biological processes, in this case skin pigmentation. I have particularly stressed the observation that phenotypic similarity is reflected on the molecular level - if two distinct (not allelic!) genetic disorders strongly resemble each other, the proteins involved are likely to interact in some way. Thus, by the simple expedient of looking closely at skin one can learn much about some very complex biology in a relatively easy way. Numerous mouse and zebrafish models greatly facilitate the study of pigmentation phenotypes and additionally provide platforms for testing treatments, or for developing drug screens. Given the crucial role that melanocytes have in protecting the skin from harmful solar radiation, as well as their

obvious cosmetic function, the basic biology of these cells deserves our undivided attention.

## **Acknowledgments**

The author is supported by grants from the Wellcome Trust, the Dystrophic Epidermolysis Bullosa Research Association (DEBRA) UK, Tenovus Scotland, The University of Dundee and the Skin Research Institute of Singapore.

## **List of abbreviations not explained in the text**

ACTH: adrenocorticotrophic hormone; ADAM10: a disintegrin and metalloprotease 10; AP1/3: adaptor protein 1/3; BLOC1-3: biogenesis of lysosome-related organelles complex 1-3 ; DCT: dopachrome tautomerase; DSRAD; GNAQ: G protein Gq $\alpha$  subunit; LYST: lysosomal trafficking regulator; MATP: membrane-associated transporter protein; MITF: microphthalmia associated transcription factor ; MYO5A: myosin 5A; PAR2: proteinase-activated receptor 2; PAX3: paired-like homeobox containing 3; PMEL17: melanocyte protein 7; RAB(n): RAS-associated protein B, there are at least 60 RABs; RAS: rat sarcoma viral oncogene homolog; SLC45A2: solute carrier family 45, member 2; SH3PXD2B: SH3 and PX domains-containing protein 2B; SOX10: SRY-related HMG-box protein 10; TYRP1: tyrosinase-related protein 1.

## **Figure legends**

Figure 1. A Somali family consisting of a mother and her two young daughters. The youngest child has type 1 oculocutaneous albinism, manifesting as a complete lack of pigmentation, which forms a dramatic contrast with her dark-skinned sib and mother.

Figure 2. A schematic illustration of known, crucial steps in neural crest specification and migration, respectively melanosome biogenesis. Genetic disorders of pigmentation are shown next to their associated protein (complex). Abbreviations are the ones used in the body text. MT: microtubules, cActin: cortical actin. The illustrations follow the order in which events are discussed in the review.

Figure 3. Griscelli syndrome type 2. A. Partial albinism with silvery hair in this child of Turkish descent, whose parents are dark-haired and have a Mediterranean skin type. B. A detail of the hair, illustrating the silvery sheen that is typical of human pigment dilution phenotypes. The child died as a result of an intracranial abscess that formed subsequent to an ear infection.

Figure 4. Reticulate hyperpigmentation of inguinal flexures in a female with dermatopathia pigmentosa reticularis. The phenotype is rather subtle and was overlooked; the patient presented for analysis of nail dystrophy, which can be part of the disorder.

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